REMARKS

The Examiner is thanked for the due consideration given the application. An expert opinion obtained at the behest of the applicant is appended to this paper.

Claims 19 and 29-36 are pending in the application. Claims 8 and 21-28 are canceled by this amendment.

No new matter is believed to be added to the application by this amendment.

Art Rejection

Claims 8, 19, 27, 29-31, 35 and 36 have been rejected under 35 USC §103(a) as being unpatentable over ELLWOOD et al. in view of HASLER et al. (U.S. Patent 6,891,037) and LANDER et al. (U.S. Patent 6,410,025). This rejection is respectfully traversed.

The present invention pertains to a method of recovering a polysaccharide from a fermentation broth that includes the utilization of anionic and/or cationic detergents. Alcohol is used to precipitate fractions.

ELLWOOD et al. pertain to the production of hyaluronic acid by the fermentation of *Streptococcus*.

ELLWOOD et al. fail to use anionic surfactant in the presence of alcohol. The anionic detergent is being removed by diafiltration before the alcohol is added. This difference reflects a difference in the function of the anionic detergent:

in ELLWOOD et al. it is added to lyse the cell and release the polysaccharide. The polysaccharide is therefore not precipitated.

In the present invention, in contrast, the anionic detergent in the presence of alcohol is used for precipitating the impurities.

Also, ELLWOOD et al. obtained an acceptable purity for its polysaccharide. The skilled person starting from ELLWOOD et al. would not be motivated to improve the purity any further.

HASLER et al. first refer to a conventional purification process, which is not extensively described therein. Subsequently, a fractional alcohol precipitation is carried out in the presence of an anionic surfactant on the obtained polysaccharide fraction. This fractional precipitation is followed by an undefined number of alcohol precipitations.

For at least the two following reasons, the skilled person would not combine ELLWOOD et al. with HASLER et al.:

1. There is no teaching in HASLER et al. to get rid of the conventional purification process.

2. The process of HASLER et al. contains at least 6 precipitations, which is an indication that it is a complicated process.

LANDER et al. also first refer to a conventional purification process that is not extensively described therein. Subsequently, LANDER et al. disclose the use of an anionic detergent. However, Lander does not disclose the simultaneous

use of an anionic detergent with alcohol (the same holds for ELLWOOD et al.). The anionic detergent is used during diafiltration to remove soluble impurities. As to the cationic detergent, it is only used to make the polysaccharide more soluble in organic solvent. In the present invention, a cationic detergent is used for precipitating the polysaccharide or part of the contaminants.

One of ordinary skill and creativity would fail to combine the references for at least the following reasons:

- The yield obtained in ELLWOOD et al. is attractive enough to keep this process and not to go looking for improvements. Neither LANDER et al. nor HASLER et al. disclose the purity of their polysaccharides fraction.

- The way anionic and cationic detergents are being used in the prior art is distinct from the way the present invention uses them. There is no teaching or inference in any of the prior art documents to use any of these detergents in another way. There is no teaching in the prior art as to how simplify classical purification processes the way the invention did.

It should also be emphasized that the present invention could also be viewed as a combination invention: it combines several features, wherein some features were known in a separate art document:

- ELLWOOD et al. disclose a multistep purification process, wherein an anionic and a cationic detergent are being used in a distinct way than in the invention,

- HASLER et al. disclose the use of an anionic detergent in the presence of alcohol as a sole purification step, and

- LANDER et al. disclose the use of a cationic detergent to precipitate the polysaccharide as a sole purification step.

However, the combination of each of these features was never disclosed nor suggested in any of these documents.

One of ordinary skill and creativity would thus fail to produce independent 19 of the present invention from a knowledge of ELLWOOD et al., HASLER et al. and LANDER et al. A *prima facie* case of unpatentability has thus not been made. Claims depending upon claim 19 are believed to be patentable for at least the above reasons.

Further, the applicant has obtained an expert opinion regarding the advantages over the conventional art. A copy of this expert opinion is attached to this paper.

In the expert opinion Michel Beurret Ph.D., of the Netherlands Vaccine Institute, compared the present invention to the applied art references and found that the present invention provides major improvements over the conventional art, including a reduction in the number of process steps, a simplification over the more complicated conventional processes, and no polishing step being required.

The present invention thus shows unexpected results over the conventional art of ELLWOOD et al., HASLER et al. and LANDER et al. and, if any unpatentability could be alleged, it is dissipated by these results.

This expert opinion can be resubmitted in the form of a Declaration, if desired.

This rejection is believed to be overcome, and withdrawal thereof is respectfully requested.

Request for Interview

The Examiner is respectfully requested to contact the applicant's representative, Robert E. Goozner, Ph.D., at 703-521-2297 in order to arrange a personal interview to discuss the patentability of the present invention.

Statement of Substance of Interview

The Examiner is thanked for graciously conducting a personal interview with the applicant's representative on July 24, 2008. During the interview the patentability of the present invention was discussed over the applied art references, especially that of ELLWOOD et al. After the interview, the Examiner prepared an interview summary. The interview summary has been reviewed, and it appears to accurately reflect the substance of the interview.

Conclusion

It is believed that the rejection has been overcome, obviated or rendered moot, and that no issues remain. The

Examiner is accordingly respectfully requested to place the application in condition for allowance and to issue a Notice of Allowability.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

- Expert opinion

Expert opinion - P207697PCT/US

- The NVI patent application for polysaccharide (PS) purification simplifies the prior art. See for example the dramatically reduced number of steps and the omitting of dangerous and/or impractical and/or costly methods (e.g. phenol extraction, chromatography). This makes it more amenable to industrial production.

- The conventional methods for bacterial PS purification, as found in the prior art, comprise a combination of the following steps:
  1. Cell inactivation by a chemical agent (e.g. formalin, phenol, detergent). Some agents can have the effect of lysis of the cells, thereby releasing their content.
  2. One cationic detergent precipitation (e.g. Cetavlon). PS is either precipitated or left in supernatant. This removes the bulk of proteins and nucleic acids.
  3. One or more alcohol precipitation(s) (e.g. ethanol). PS is precipitated. Small impurities are not precipitated. This further purifies the PS.
  4. One or more fractional alcohol precipitations (e.g. ethanol). These include two distinct steps: PS is left in supernatant in the first step, then precipitated in the second step. This removes some more impurities, which are precipitated in the first step, while PS is left in supernatant.
  5. Additional "polishing" step (e.g. activated charcoal, phenol extraction, enzyme treatment, hydroxylapatite adsorption, chromatography, etc.) This removes the last traces of contaminants (including e.g. endotoxin).
  6. Centrifugation (normal speed, high speed, or continuous flow) is used throughout to separate solid from liquid material. It can be replaced by normal filtration, which can be more advantageous for large scale production.
  7. Diafiltration is also used to remove very small soluble contaminants (from the culture medium, the bacteria, or the extraction process itself). It is also used for buffer exchange, i.e. to replace the fluid in which the PS is dissolved by another fluid (e.g. phosphate buffer by water). Unlike with "normal" filtration, there are here no solids to be separated from liquid.

- Multiplying and repeating the purification steps will result in a very pure product. Therefore, the challenge is to design a process with a small number of steps, yet that still affords a pure product. This is achieved by the NVI's patent, as illustrated by the purification of *Haemophilus influenzae* (Hib) PS.

- The order in which the different steps are performed can also affect the outcome, namely the purity of the final product (PS). For example, the cationic detergent precipitation can be placed almost anywhere (before, in between, or after the alcohol precipitations).

- **Ellwood** 1996 (US 5,563,051)
  - Process description:
    1. Formalin: added to cell culture; kills the cells.
    2. Anionic detergent (SDS): added to cell culture; lyses the cells and releases the PS (no precipitation).
    3. Diafiltration (water): removes small soluble contaminants, including the anionic detergent (cf. col.4-ln.39 and ex.1-col.6-ln.19).
    4. Salt and pH adjustment.
    5. Optional cationic detergent precipitation (cetyl pyridinium chloride): if a medical grade product is desired; removes nucleic acids; PS is left in supernatant.
    6. Several alcohol precipitations (isopropyl alcohol): removes more contaminants, PS is precipitated (cf. ex.1-col.6-ln.34-42: two precipitations).
    7. Removal of alcohol by drying.
  - Major differences with NVI's patent:
    - Number of steps: at least 3 precipitation steps. Total: at least 3 precipitation steps.
    - **Product is always hyaluronic acid** (cf. col.1-ln.6-7); **no evidence/claim that process can be applied to other bacterial PS.**
    - The SDS step causes cell lysis, which releases large amounts of contaminants such as nucleic acids. Fortunately, hyaluronic acid has a very high density of negative charges which can facilitate its separation from nucleic acids. On the other hand, PS from Hib and many other pathogenic bacteria have a lower density of negative charges, which makes them more similar to nucleic acids, and therefore more difficult to separate from the impurities.
    - In the examples given, hyaluronic acid is extracted from Gram-positive bacteria (*Streptococcus equi*). These do not contain endotoxin (lipopolysaccharide). This is yet another aspect showing that this purification is relatively simple. On the other hand, many bacterial PS of interest for vaccine use are extracted from Gram-negative bacteria (e.g. Hib, *Neisseria meningitidis*, etc.). These contain a lot of endotoxin which is present in the original PS. Both compound are notoriously difficult to separate from each other.
    - **Anionic detergent: no simultaneous use with alcohol, since the anionic detergent is removed by diafiltration** before the first alcohol precipitation. It follows logically that **no anionic surfactant is present when the alcohol is added. This use of anionic detergent bears no resemblance to that in NVI's patent.**

- **Cationic detergent: no precipitation of PS,** since only the nucleic acid impurities are precipitated.
- **No use of fractional alcohol precipitations:** PS is always precipitated.

- **Hassler** 2005 (US 6,891,037)
  - Process description:
    1. Conventional purification without phenol extraction: not described (cf. ex.1-col.4-ln.39).
    2. One fractional alcohol precipitation with anionic detergent (ethanol and SDS). PS is left in supernatant in the first step, then precipitated in the second step. This removes impurities (mainly endotoxin), which are precipitated in the first step, while PS is left in supernatant.
    3. Several alcohol precipitations (ethanol): removes SDS impurities, PS is precipitated; undetermined number. (cf. ex.1-col.4-ln.51-55)
    4. Removal of alcohol not mentioned.
  - Major differences with NVI's patent:
    - Number of steps: the additional purification process includes at least 4 precipitation steps, to be added to the conventional purification (undetermined number of steps: at least 2). **Total: at least 6 precipitation steps.**
    - **No mention of how to simplify the conventional purification** augmented by the additional purification step.

- **Lander** 2002 (US 6,410,025)
  - Process description:
    1. Conventional purification: not described (cf. ex.1-col.4-ln.37-40).
    2. One cationic detergent precipitation (cetyl pyridinium chloride or Cetavlon): PS is precipitated; this also removes impurities (cf. col.2-ln.58-63).
    3. PS dissolution in organic solvent (DMSO or DMF) (cf. col.2-ln.54-57).
    4. PS derivatization and conjugation to protein carrier (OMPC).
    5. Diafiltration (phosphate buffer). This removes impurities (unreacted PS, excess reagents); PS conjugate is left in concentrate (cf. col.4-ln.16-19).
    6. Diafiltration with anionic detergent (buffer containing DOC). This removes soluble impurities (mainly endotoxin released by conjugation); PS conjugate is left in concentrate (cf. col.4-ln.19-22).
    7. Diafiltration (saline then water)
  - Major differences with NVI's patent:
    - Number of steps: the additional purification process includes 1 precipitation step, to be added to the conventional purification (undetermined number of steps: at least 6-7 as described in

Marburg 1987 [US 4,695,624] and Kniskern 1992 [EP 0 497 525 A2], both from the same vaccine producer). **Total: at least 7-8 precipitation steps.**

- **No mention of how to simplify the conventional purification** (ex.1-col.4-ln.37-40) augmented by the additional purification step.
- **Cationic detergent: the main goal is to make the PS more soluble in organic solvents** (cf. col1-ln.41-51 & col.2-ln.54-57). Removal of impurities by precipitation of the PS is a further advantage of the reaction (cf. col.2-ln.58-63).
- **Anionic detergent: no simultaneous use with alcohol,** since the anionic detergent is used during diafiltration to remove soluble impurities. **This use of anionic detergent bears no resemblance to that in NVI's patent.**
- In the three examples given, PS from Gram-positive bacteria (*Streptococcus pneumoniae*) is purified. These do not contain endotoxin (lipopolysaccharide). On the other hand, the protein carrier (OMPC = outer membrane protein complex, from *Neisseria meningitidis*, a Gram-negative bacteria; cf. EP 0 497 525 A2) contains a lot of endotoxin which is released during the conjugation process (cf. col.4-ln.19-22). Logically, this implies that the endotoxin was not present in the original PS but was later added during the conjugation process. **The anionic detergent purification step is therefore clearly designed to correct an imperfection of the conjugation process** and not to purify a crude PS fraction.
- **No use of (fractional) alcohol precipitations.**

▪ Suggested changes to NVI patent application

- The use of anionic detergent and alcohol below the concentration at which the PS precipitates was mentioned in old claim 7. This has now been included as introduction to claim 8, but it does not add any valuable information that is not already contained in the rest of claim 8. It is also confusing since it does not mention simultaneity of use. If this part is removed, claim 8 becomes identical to claim 19. Claim 8 should be removed and all other claims made dependant on claim 19 (instead of 7 or 8).
- The use of cationic detergent makes mention of the precipitation of either the PS or "part of the contaminants". This last part could be removed from claims 8 & 19, since it is useless in the current process. It is also anticipated by Ellwood. Problem: this precipitation of contaminants is mentioned in two additional embodiments in the description (cf. p.7-ln.30-32 & p.8-ln.1-4), but these are not illustrated by examples. Example 3 follows the main embodiment (cf. p.7-ln.26-29 & p.14 & fig.2).
- Deleted claims (8): 8, 29-35
- Remaining claims (10): 19, 21-28, 36

- **NVI** patent
  - Process description:
    1. Formalin: added to cell culture; kills the cells. No cell lysis.
    2. Cationic detergent precipitation (Cetavlon): PS is precipitated; removes nucleic acids
    3. Alcohol precipitation (ethanol): removes more contaminants, PS is precipitated.
    4. One fractional alcohol precipitation with anionic detergent (ethanol and DOC). PS is left in supernatant in the first step, then precipitated in the second step. This removes impurities (mainly endotoxin), which are precipitated in the first step, while PS is left in supernatant.
    5. Removal of small contaminants (e.g. alcohol) by diafiltration.
  - Major improvements over conventional methods:
    - **Number of steps: only 4 precipitation steps.**
    - **Simplifiifation** of conventional processes.
    - **No polishing step required.**

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